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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte MARC PREAUDAT, CHIKASHI TOKUDA,
and LAURENCE JACQUEMART

Appeal 2009-012075[1]
Application 10/522,909
Technology Center 1600

Before ERIC GRIMES, DEMETRA J. MILLS, and MELANIE L. McCOLLUM, *Administrative Patent Judges*.

McCOLLUM, *Administrative Patent Judge*.

DECISION ON APPEAL[2]

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[1] Oral Hearing held June 24, 2010.

[2] The two-month time period for filing an appeal or commencing a civil action, as recited in 37 C.F.R. § 1.304, or for filing a request for rehearing, as recited in 37 C.F.R. § 41.52, begins to run from the "MAIL DATE" (paper delivery mode) or the "NOTIFICATION DATE" (electronic delivery mode) shown on the PTOL-90A cover letter attached to this decision.

This is an appeal under 35 U.S.C. § 134 involving claims to an enzyme activity determination method. The Examiner has rejected the claims on appeal as obvious. We have jurisdiction under 35 U.S.C. § 6(b). We reverse.

STATEMENT OF THE CASE

The Specification states that the "FRET (fluorescence resonance energy transfer) phenomenon allows homogeneous time-resolved measurement of fluorescence" (Spec. 4). The Specification discloses "an assay for endoglycosidase activity based on homogeneous time-resolved measurement of fluorescence resulting from an energy transfer between a donor fluorescent compound and an acceptor fluorescent compound, which are attached to the substrate" (*id.*) The Specification indicates that "the distance separating the first and second fluorescent compound must both allow the energy transfer to take place and allow the cleavage of the substrate to effectively bring about a distancing of the two fluorescent compounds" (*id.* at 5).

Claims 1, 2, and 5-12 are on appeal. Claims 3, 4, and 13-18 are also pending but have been withdrawn from consideration by the Examiner. (App. Br. 1.) We will focus on claim 1, the only independent claim on appeal, which reads as follows:

1. A method for determining endoglycosidase enzyme activity in a sample, comprising:
   - i. bringing a substrate which can be cleaved by said endoglycosidase into contact with said sample, and
   - ii. measuring a change in the amount of intact substrate, a decrease in the amount of this substrate being representative of endoglycosidase activity in the sample,

wherein the substrate is directly or indirectly labeled with a first donor compound and with a second acceptor compound, and the amount of intact substrate is determined by measuring a signal emitted by the acceptor compound, this signal resulting from a transfer, via a close proximity effect, between the donor and the acceptor.

Claims 1, 2, and 5-12 stand rejected under 35 U.S.C. § 103(a) as obvious over Bazin[3] in view of Nicolson[4] (Ans. 2).

The Examiner relies on Bazin for teaching "FRET-based enzyme assays using a class of fluorescent complexes, the rare earth cryptate, as a fluorescent donor with allophycocyanin as acceptor for probing molecular interactions in biology" (*id.* at 2-3). The Examiner relies on Nicolson for teaching "a method for determining endoglycosidase . . . enzyme activity in a sample by labeling a substrate at one or more sites" (*id.* at 3). The Examiner concludes that it would have been obvious "to modify the method of Bazin[] to determine endoglycosidase enzyme activity according to Nicolson because Bazin teaches that the FRET-based assay is designed to reveal substrate modification in enzyme mediated reactions that cleave . . . substrates" and "specifically described that their FRET-based method demonstrated ease in labeling different type of molecules (thus including . . . sugar substrates if they are involved in the particular enzyme activity under investigation)" (*id.*).

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[3] H. Bazin et al., *Homogeneous time resolved fluorescence resonance energy transfer using rare earth cryptates as a tool for probing molecular interactions in biology*, 57 SPECTROCHIMICA ACTA PART A 2197-2211 (2001).
[4] Nicolson et al., US 4,859,581, Aug. 22, 1989.

ISSUE

Does the evidence support the Examiner's conclusion that it would have been obvious to use Bazin's FRET-based technology in Nicolson's endoglycosidase enzymatic activity assay?

FINDINGS OF FACT

1. Bazin discloses a "homogenous assay technology using time resolved fluorescence and fluorescence energy transfer" in which a "class of fluorescent complexes, the cryptates, have been used as fluorescent donor with cross-linked allophycocyanin as acceptor" (Bazin, Abstract).

2. Specifically, Bazin discloses using TBS Eu^{3+} as a cryptate donor and XL665 as an acceptor (*id.* at 2200-2202).

3. Bazin also discloses that "[e]nzyme mediated reactions that cleave, synthesize or modify substrates represent a significant group of target in biology" and that FRET "assays were designed to reveal substrate modifications in different domains" (*id.* at 2206).

4. In particular, Bazin describes a protease assay in which:

> A peptide containing a sequence recognized by the protease was labeled on one side with XL665, and on the other side with biotin. Upon the addition of TBP Eu^{3+} labeled streptavidin, a specific long-lived signal was measured at 665 nm. In the presence of the protease, which cleaves the peptide, the signal decreases.

(*Id.*)

5. Bazin also discloses that there is a "demonstrated ease in labeling different type[s] of molecules, peptides and oligonucleotides with TBP Eu^{3+} and XL665" (*id.* at 2209).

6. Nicolson relates to "an assay for endoglycosidase enzymic [sic] activity and a labeled substrate for use in such an assay" (Nicolson, col. 1, ll. 10-13).

7. In particular, Nicolson discloses a "solid phase substrate which yields soluble labeled products upon hydrolysis by a glycosaminoglycan endoglycosidase[, the] . . . substrate compris[ing] glycosaminoglycan bearing labeled substances" (*id.* at Abstract).

8. Nicolson also discloses that the solid phase substrate "may comprise as the glycosaminoglycan: hyaluronic acid, chondroitin 4-sulfate, chondroitin 6-sulfate, dermatan sulfate, keratan sulfate, heparan sulfate, heparin, or combinations thereof" (*id.* at col. 6, ll. 9-13).

9. To produce the substrate, Nicolson discloses a method comprising: "at least partially N-desulfating or N-deacetylating a glycosaminoglycan; labeling at least partially N-deacetylated or N-desulfated glycosaminoglycan to produce labeled glycosaminoglycan; completely N-acylating the labeled glycosaminoglycan. . . ." (*Id.* at col. 4, ll. 58-63.)

10. To label the substrate, Nicolson discloses using a substance yielding a detectable signal, such as a radioisotopic label, a fluorescent label, or an enzymatic label (*id.* at col. 5, ll. 3-7).

11. In addition, Nicolson discloses:

> The glycosaminoglycans generally have their amine functions either sulfated or acetylated. After at least partial N-desulfation or N-deacetylation, for example, the resultant primary amino groups on the glycosaminoglycan are available for labelling. . . . A label is then covalently attached to at least some of the

> free amine groups. Remaining free amine groups of the labeled glycosaminoglycan are then acylated.

(*Id.* at col. 6, l. 64, to col. 7, l. 13.)

12. Nicolson also discloses that "[a]mino group labeling may be accomplished by coupling a measurable compound or active protein to at least a few of the amino groups" (*id.* at col. 7, ll. 20-22).

13. In addition, Nicolson discloses:

> In preliminary experiments, a partially N-desulfated heparan sulfate was coupled in a 1:1 ratio to fluoroscein isothiocyanate. This fluorescein labeled derivative was found to be a good substrate for melanoma heparanase. It is contemplated that up to a 10:1 ratio of fluorescein to HS may be produced and serve as a haparanase substrate.

(*Id.* at col. 7, ll. 36-42.)

PRINCIPLE OF LAW

A claim "composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art." *KSR Int'l v. Teleflex Inc.*, 550 U.S. 398, 418 (2007).

ANALYSIS

Bazin discloses an enzyme assay that uses a peptide substrate recognized by the enzyme, specifically a protease, the peptide substrate being labeled on one side with an acceptor, XL665, and indirectly labeled on the other side with a donor, TBS Eu^{3+} (Findings of Fact (FF) 1-4). Bazin discloses that, "[i]n the presence of the protease, which cleaves the peptide, the signal decreases" (FF 4). Bazin also discloses that there is a "demonstrated ease in labeling different type[s] of molecules, peptides and oligonucleotides with TBP Eu^{3+} and XL665" (FF 5). However, the

Examiner acknowledges that Bazin does not disclose that the enzyme is endoglycosidase (Ans. 3), nor does the Examiner point to any teaching in Bazin of a substrate comprising a glycosaminoglycan or other polysaccharide.

Nicolson relates to "an assay for endoglycosidase enzymic [sic] activity and a labeled substrate for use in such an assay" (FF 6). Nicolson's substrate comprises a labeled glycosaminoglycan, such as "hyaluronic acid, chondroitin 4-sulfate, chondroitin 6-sulfate, dermatan sulfate, keratan sulfate, heparan sulfate, heparin, or combinations thereof" (FF 8). As noted by Appellants, glycosaminoglycans, such as heparan sulfate, are complicated and varied molecules (App. Br. 4-6; *see also* Spec 3: 24-31 & 6: 20-32). Nicolson discloses a technique for labeling these molecules (FF 9-13). However, the Examiner has not provided adequate reasoning to support the conclusion that Nicolson's technique could be used to provide Bazin's FRET labels at appropriates distances on either side of the cleavage site, as described in Bazin. Therefore, we agree with Appellants that the Examiner has not set forth a prima facie case of obviousness.

CONCLUSION

The evidence does not support the Examiner's conclusion that it would have been obvious to use Bazin's FRET-based technology in Nicolson's endoglycosidase enzymatic activity assay. We therefore reverse the obviousness rejection.

REVERSED

alw

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